REMARKS

The Specification has been amended per the Examiner's request: the hyperlink was removed and replaced with the name of the Website which is not browser-executable. Therefore no new matter has been introduced with this amendment. Claims 23-33 which were withdrawn previously as being drawn to a non-elected invention are now canceled. Additionally, claims 3, 10 and 38 have been canceled. Claims 14, 15, 16 and 34 have been amended. Support for the amendments can be found in Claims 1 and 12. Therefore, no new matter has been introduced with these amendments. The following addresses the substance of the Office Action.

Ground(s) of rejection

The Examiner has indicated that this action had been made final because the new ground(s) of rejection were necessitated by the Applicant's amendment. As discussed below, Applicants maintain that Saiki is not relevant to the pending claims. Applicants further maintain that the amendments introduced by the Applicant on April 8, 2004 did not impact the relevancy of Saiki to the claims. In particular, the amendment introduced by the Applicant on April 8, 2004 amended the length of the double-stranded DNA spacer from "at least 20" to "between about 50 and about 250" base pairs. Applicants maintain that this amendment did not necessitate the new grounds of rejection based on Saiki. Accordingly, Applicants respectfully requests withdrawal of the finality of the current Office Action (see MPEP 706.07(a)).

Claim Objections

The Examiner requested correction of Claim 34, due to its dependence on later Claim 36. The Applicant has amended Claim 34 to now properly depend on Claim 12.

Non-obviousness

The Examiner has rejected Claims 1-8, 10-15, 17-22, 34, 36 and 38 under 35 U.S.C. §103(a) as being allegedly unpatentable over Peterson et al. (WO 95/30026) in view of Saiki et al. (WO 89/11548). More specifically, the Examiner believes that one of ordinary skill in the art would have been motivated at the time the invention was made to apply Saiki et al. teaching of using spacer sequences to Peterson et al. method of screening transcriptional factors in order to increase hybridization efficiency by moving the hybridizing region of probe away from support to increase the efficiency of binding with target. Applicant respectfully disagrees.

To establish a *prima facie* case of obviousness, the PTO must cite one or more references that provide some suggestion or motivation to modify the references to achieve the claimed

invention, provide a reasonable expectation of success to achieve the claimed invention, and finally, the cited art must teach or suggest all the claim limitations. *In re Vaeck*, 947 F.2d 488 (Fed. Cir. 1991). Here, the cited art either taken alone or in combination, fails to provide any of the required factors.

Peterson et al. describes assays for screening for drugs which interfere with sequence-specific protein-DNA binding. Peterson et al. neither suggest nor mention the use of a spacer. Peterson does have, on average, 10 bp in front of the transcription factor binding site. However, Peterson does not disclose or suggests spacers between about 50 and about 250 bp as recited in the independent Claim 1. As described in the accompanying Declaration, use of spacers between about 50 and about 250 bp in length provides enhanced signal levels relative to the levels observed when the binding site is separated from the support by shorter sequences with a length similar to those described in Peterson. These enhanced signal levels are particularly important in the context of microarrays, in which the binding of multiple factors to their recognition sites is evaluated under uniform binding conditions which may be suboptimal for the binding of at least some of the factors. In such situations, it is desirable to enhance signal levels as much as possible since the suboptimal binding conditions may result in a lower degree of binding than would be observed under optimal conditions. In this regard, it is important to note that the methods of Peterson are performed on multiwell plates as opposed to the microarrays utilized in the present methods. Thus, use of 10 bp sequences before the binding site as disclosed in Peterson is insufficient for use in the context of microarrays.

As stated in the Inventor's Declaration under 37 C.F.R. §1.132 submitted herewith, spacers between about 50 bp and about 250 bp provide advantages in binding efficiency. As described in the Declaration, the inventors first performed quantitation of signals resulting from NF-kB binding to recognition sites linked to spacers of various sizes: from 6 bp (close to conditions used by Peterson et al., where the spacer was 8 bp long) to 100 bp. Signal strengths for NF-kB binding were maximum with a spacer of 100 bp.

As described in the accompanying Declaration, similar experiments revealed that increasing spacer lengths increased signal intensities for 4 transcription factors, Elk-1, c-Myk, STAT1 and STAT3, in addition to NF-kB. In addition, as described in the accompanying Declaration, it was possible to detect and quantify 5 different transcriptional factors with accuracy on a microarray under the same binding conditions. Furthermore, contrary to detection

of NFκB factor which was still detectable with the use of a small spacer (6 bp), some factors were hardly detectable (STAT3), or not detected at all (Elk-1) with a small spacer. The inventors also found that the signals measured with spacers below about 50 bp may not increase linearly with the spacer size. Therefore, high variability in signal detection using short (6 bp) spacers is totally incompatible with the simultaneous analysis of more than one factor as claimed in the present invention. Because the binding conditions would be uniform on the array, it is likely that the binding conditions (salt concentrations, temperature, etc.) would be less than optimal for each of transcription factors. In view of the suboptimal binding conditions, the optimal spacer size provides important advantages in detecting and quantifying transcription factor binding. The inventors show in the Declaration that the signals for all the tested transcription factors when tested simultaneously on a single array were maximal when the spacers were between about 50 and 150 basepairs. Therefore, the spacers between about 50 and about 250 base pairs are not obvious in view of Peterson et al.

Saiki et al. (WO 89/11548) teach an improved nucleic acid hybridization assay reagent, which uses a single-stranded (ss) DNA tail in order to move the hybridization region of a probe away from the support and thereby improve the hybridization efficiency between two single-stranded nucleic acid sequences. Saiki et al. neither suggest nor mention the use of a double-stranded (ds) DNA spacer connected with a specific sequence being able to bind one or more transcriptional factors or any other protein.

Applicants maintain that Saiki is not relevant to the pending claims because it relates to single-stranded tails as opposed to the double-stranded spacers recited in the pending claims and because it relates to probes for use in single-stranded DNA(ssDNA)-single stranded DNA hybridization as opposed to binding between a transcription factor and a double-stranded DNA sequence. Applicants note that the tails in Saiki, are generated by adding nucleotides to the end of the hybridization probe using enzymes or commercially available synthesizers (see page 15, lines 3-5 of Saiki). These methodologies result in single-stranded tails as opposed to double-stranded spacers as recited in the pending claims. There is no teaching or suggestion in Saiki of using double-stranded spacers.

Furthermore, Applicants maintain that Saiki is not relevant to the pending claims because the kinetics and nature of ssDNA-ssDNA binding is completely different from the kinetics and nature of binding of a transcription factor to a double stranded target sequence. As stated in the

accompanying Inventor's Declaration, the binding mode of ssDNA-ssDNA is in no way comparable to that of transcriptional factor-dsDNA, due to the completely different nature of the interacting partners. First, DNA and proteins are different classes of molecules having different physical and chemical properties. While ssDNA is made of a succession of 4 types of nucleotides which form a uniform molecule with a well defined structure when present in double-stranded form, proteins are made of 20 different amino acids and exhibit a large variety of properties (hydrophilic-hydrophobic, acido-basic, ...), according to their composition and their possible post-translational modifications. This is of particular importance in the context of transcriptional factors, as many of them require phosphorylation or association events to become functionally active and acquire/retain a DNA-binding capacity. Accordingly, each transcriptional factor exhibits unique structural and therefore binding properties, that are very different for one another. The complexity of transcriptional factor-dsDNA interactions is further increased by the absolute requirement for the factor to exhibit its native conformation. Indeed, the functionality of the DNA binding motifs of transcriptional factors highly depends on their surrounding structural context.

As stated in the accompanying Declaration, the nature and kinetics of the interactions between ssDNA-ssDNA and transcriptional factor-dsDNA are also different. The ssDNA-ssDNA interaction is mediated through hydrogen bonds between the nucleotide bases of each strand that are favored at high temperature and salt concentration. Transcriptional factor-dsDNA interactions are mediated by much more complex interactions, involving salt bridges, hydrogen bonds, hydrophobic interactions and metallic ion chelation, and must take place at moderate temperature to avoid the denaturation of the protein. Another major difference resides in the size of the interacting partners. While ssDNA molecules exhibit some structural flexibility which allows the interaction of any internal sequence with an immobilized complementary sequence, the globular nature of transcriptional factors as well as their important size render their interaction with dsDNA target sequences much less flexible.

Thus, because the hybridization of single stranded nucleic acids is vastly different in kinetics and complexity than protein DNA interactions, one skilled in the art would not look to Saiki for guidance on assessing protein DNA interactions.

Furthermore, there is no teaching or suggestion in Saiki that double-stranded spacers would provide benefits in the context of DNA protein interactions as recited in the present

claims. In the present invention, there are three levels of structural limitations to circumvent in order to obtain a specific binding and detection/quantification of one or more transcriptional factors. Firstly, the transcriptional factors have to bind their consensus dsDNA sequences fixed upon the solid support despite their large size and low flexibility. Secondly, the complexes have to be recognized, for example, by an antibody to identify and quantify the signal resulting from the binding of the transcriptional factors with their consensus sequences, imposing native binding conditions. Thirdly, multiple transcriptional factors have to be detectable simultaneously on the same support despite their different DNA binding properties.

These difficulties are not mentioned by Saiki et al. nor are solutions proposed. In addition, Applicants note that Saiki did not teach or suggest that spacers in the size ranges recited in the pending claims are preferred. In fact, the preferred embodiment of Saiki et al. employs tails at least 400 nucleotides in length (page 17, line 24 and examples) which is out of the range described in claim 1 of the present invention (about 50-about 250 bp). Moreover, in Saiki et al. "the exact number of nucleotides is not critical" (page 17, lines 25-26), and "larger tails are doubly preferred" (page 18, lines 10-11), which teaches away from the present invention.

In addition, the nature of the tails used by Saiki et al. also does not suit the method described in the present invention. Saiki et al. use ssDNA spacers, which are preferably composed of repeated nucleotides (homopolymers; page 17, line 13 and examples), and argue that the use of heteropolymers tails may be a problem (page 17, lines 31-32 to page 18, lines 1-4).

Furthermore, the homopolymer ssDNA tail of Saiki et al. is synthesized by enzymatic reaction. The terminal transferase (Tdt) tails the probe at the 3' end with dTTP (page 24, line 20). This method is not compatible with the synthesis of dsDNA spacer as required in the present invention.

As stated in the Inventor's Declaration under 37 C.F.R. §1.132 submitted herewith, the present invention employs spacers corresponding to or containing at least a dsDNA nucleotide sequence with a specific length (about 50- about 250 bp), preferably of heteropolymeric composition (see examples in the Specification as filed).

Finally, Saiki et al. suggest that diverse sets of oligonucleotides specific for different sequences can be immobilized on the same membrane (page 24, lines 3-5). However, the Example 1 of Saiki et al. shows that the array is not formed in a miniaturized format since 100 µl of probe are spotted onto the filters. This volume is about 10^5 times higher than the volume

currently delivered on microarrays and will not allow reaching a density of at least 4 spots/cm^2 of solid support surface as in claim 1 of the invention, because of the solution spreading. It is important to note that the kinetics of hybridization would differ significantly between a macroarray and a microarray due to the different amount of immobilized probe. This further highlights the difference between the present invention and the method of Saiki et al.

Therefore, Peterson et al. and Saiki et al. references fail to support *prima facie* case of obviousness. These references both fail because neither provides the requisite motivation to combine, the reasonable expectation of success, or teaches all the limitations of the claimed invention. Because of these deficiencies, Applicants submit the PTO has failed to articulate a *prima facie* case of obviousness, and as such, request that the present rejection of Claims 1-8, 10-15, 17-22, 34, 36 and 38 should be withdrawn.

Allowable subject matter

The Examiner has objected to Claim 16 for depending on rejected claim, but indicated that there is no prior art that teach or suggest the method where the transcriptase is HIV integrase. The Applicant has rewritten Claim 16 in independent form incorporating all the limitations of the preceding claims on which it was previously dependent. Therefore, the currently amended Claim 16 should be allowable now.

CONCLUSION

For all the foregoing reasons, it is respectfully submitted that the rejections set forth in the outstanding Office Action are inapplicable to the present claims. Accordingly, Applicants request the expeditious allowance of the pending claims.

The undersigned has made a good faith effort to respond to all of the rejections in the case and to place the claims in condition for immediate allowance. Nevertheless, if any undeveloped issues remain or if any issues require clarification, the Examiner is respectfully requested to call the undersigned at (619) 687-8633 (direct line), to discuss such issues.

Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. 11-1410.

Respectfully submitted,

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